(12) UK Patent Application (19) GB (11) 2 051 575 A

(21) Application No 8021943
(22) Date of filing
4 Jul 1980
(30) Priority data
(31) 7917451
(32) 5 Jul 1979
(33) France (FR)
(43) Application published
21 Jan 1981
(51) INT CL³ A61K 35/78
(52) Domestic classification
A5B 170 180 303 30X
30Y 38Y 391 E H
(56) Documents cited
GB 1106133
(58) Field of search
A5B
(71) Applicant
Synthelabo
1 Avenue de Villars
75341 Paris
France
(72) Inventors
Pierre Bernard
Guy Balansard
(74) Agents
Messrs J A Kemp & Co

(54) Pharmaceutical compositions containing climbing ivy extracts

(57) Extracts of climbing ivy containing at least 60% of hederasaponin C or comprising alpha-hederin are useful in treating animals (including humans, sheep and cattle) against parasites and fungi. The extracts can be prepared by leaching the ivy with acetone or equivalent, extracting the dried solids with pure methanol, precipitating a solid by adding diethyl ether or equivalent to the methanolic solution and optionally chromatographing the product on alumina with methanol as eluant to increase the hederasaponin C context of the extract.

GB 2 051 575 A

SPECIFICATION

Pharmaceutical compositions containing climbing ivy extracts

The present invention relates to pharmaceutical compositions containing climbing ivy extracts and to the preparation of extracts based on hederasaponin C from ivy.

Extracts of ivy (Hedera) and more particularly of climbing ivy (Hedera helix) have already been prepared by extraction with water and/or alcohol.

The present invention relates to the preparation of an extract containing 60% of saponins from climbing ivy, and more particularly to the preparation of a purified saponin extract containing 90% of hederasaponin C, and finally to the preparation of α-hederin.

The invention includes a process for the preparation of a climbing ivy extract containing at least 60% by weight of hederasaponin C, which process comprises leaching powdered ivy with acetone, or a solvent having a dielectric constant about the same as that of acetone, drying the powder thus leached, extracting the dried powder with substantially pure methanol, to give a solution of extracted material in methanol, concentrating the said solution, treating the resultant concentrate with neutral activated charcoal to absorb impurities thereon, separating the charcoal from the solution, adding diethyl ether, or a mixture of organic liquids which has a polarity about the same as that of diethyl ether, to the resultant concentrate in order to precipitate saponins and recovering a precipitate comprising a climbing ivy extract containing 60% by weight of hederasaponin C, and if desired chromatographing this extract on alumina with methanol as eluant, to give a climbing ivy extract containing more than 60%, preferably at least 90%, by weight of hederasaponin C. Preferably the precipitate containing 60% by weight of hederasaponin C is recovered by separating the precipitate from the liquid of the concentrate, taking up the precipitate in substantially pure methanol and separating solids from the methanolic liquid.

The invention also provides a method of treating a non-human animal against a fungal or parasitic infection which comprises administering to the animal a therapeutically effective amount of a climbing ivy extract containing at least about 60% by weight of hederasaponin C or comprising alpha-hederin. Preferably the extract contains about 60% or at least about 90% by weight of hederasaponin C. The parasitic activity against intestinal worms, i.e. anthelmintic activity, is especially valuable. The method of the invention is useful, for example, in treating sheep or cattle for flukes, especially liver fluke and lancet fluke.

The invention further includes a pharmaceutical composition comprising a climbing ivy extract containing at least 60% by weight of hederasaponin C or comprising alpha-hederin, in association with a pharmaceutically acceptable excipient. Particularly useful such compositions include sugar-coated pills, solutions, especially sterile injectable and/or lyophilised solutions, ointments for topical application and eye-washes.

Diethyl ether or a mixture of solvents having a polarity which is identical to that of ether, for example a mixture of chloroform and ethyl acetate, is then added to the filtrate in order to precipitate the saponin extract. This extract is taken up in pure methanol, filtered off and dried under reduced pressure or by spray-drying.

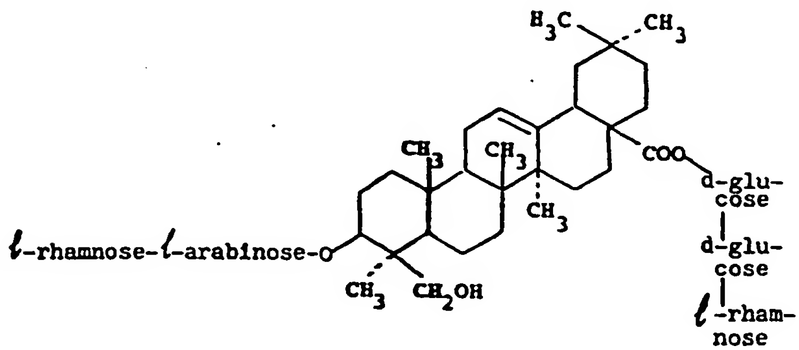
The product thus obtained is a saponin complex containing about 60% of hederasaponin C.

This yellow-coloured complex is very soluble in water and methanol and sparingly soluble in ethanol.

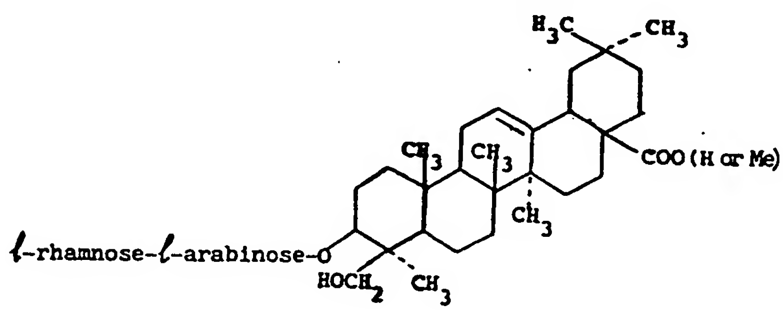
In thin layer chromatography on silica gel, using a 65/35/10 mixture of benzene, methanol and acetic acid as the solvent system and a 1% strength solution of vanillin in sulphuric acid as the developer, various coloured spots are revealed, including, in particular, a black-brown coloured spot for hederasaponin C.

In accordance with the process of the invention, an extract containing at least 60% preferably of at least 90% of hederasaponin C is prepared in a second stage. For this purpose, the previous extract containing 60% of hederasaponin C is treated on an alumina column, elution being carried out with pure methanol.

The eluate collected contains a minimum of 90% of hederasaponin C of the formula



In accordance with the process of the invention, the final step consists in preparing α-hederin or its alkali metal salts from the previous extract by saponification with sodium hydroxide or potassium hydroxide; α-hederin has the formula



The three substances obtained at each stage of the process of the invention have been studied in respect of their antifungal and antiparasitic activity in vitro and in vivo.

The following example illustrates the process of the invention.

EXAMPLE

1. 25 kg of powdered climbing ivy are treated with 120 litres of acetone. The mixture is concentrated and the concentrate is dried. This yields a dark green viscous mass weighing about 1,000 g, which is dried.

110 litres of pure methanol are added. The mixture is concentrated to 25 litres under reduced pressure. The concentrate is stirred with 100 to 200 g of neutral activated charcoal. The mixture is filtered. The filtrate is concentrated to about 10 litres under reduced pressure. 20 litres of ethyl ether are added to the 10 litres of filtrate. This yields a precipitate P_1. The filtrate is concentrated to 6 litres, and 20 litres of ethyl ether are added. A precipitate P_2 is formed and this is combined with the precipitate P_1. The precipitates are taken up in 10 litres of pure methanol, filtered off and dried under reduced pressure. This yields 1.5 to 2 kg of extract containing 60% of hederasaponin C.

2. The separation on an alumina column in order to obtain the extract containing 90% of hederasaponin C is carried out in the following manner:

1 kg of W_{200} neutral alumina is dispersed in pure methanol and then introduced into the column. Elution is carried out with pure methanol until the eluate is perfectly clear. 300 g of the previous extract, diluted in 2 litres of pure methanol, are then introduced. Elution is carried out with pure methanol. 4 litres of eluate are collected and evaporated under reduced pressure. This yields 190 g of extract containing a minimum of 90% of hederasaponin C.

3. The extract containing 90% of hederasaponin C is treated, in an aqueous medium, with 2N NaOH or KOH under the action of heat for 1 hour.

The mixture is acidified and the precipitate is washed. It is taken up in pure methanol, filtered off and dried.

This yields α-hederin.

The climbing ivy extracts obtained in accordance with the process of the invention, namely the extract containing 60% of hederasaponin C, the extract containing 90% of hederasaponin C, and α-hederin, have proved to be active as antiparasitic and antifungal agents.

The toxicity of the extracts was determined on mice by intraperitoneal administration.

The LD 50 (24 hours) varies from 2,000 mg to 3,200 mg. The LD 50 (8 days) varies from 1,500 mg to 2,500 mg.

Antiparasitic activity

The anthelmintic activity and the protozoicidal activity of the extracts of the invention were studied.

1. *The anthelmintic activity* with respect to cestodes, nematodes and trematodes was determined in vitro and in vivo.

1.1 The activity with respect to cestodes was studied on mouse Taenia:

In vitro experiment:

The Hymenolepis nana, fraterna variety, are recovered by dissecting the small intestine of mice infested with the parasites, and are placed in a Sen and Hawking medium in an oven at 37°.

The experiment consists in bringing various dilutions of the products to be tested into contact with the worms, which have been kept alive, and in determining the lethal dose after 24 hours.

Comparison is made with 1 batch of untreated worms, which should survive several days, and 1 batch of worms treated with reference products, namely helenin and santonin.

In vivo experiment:

This consists in causing the mouse infested with the parasites to ingest varying doses of products to be tested, and in monitoring the Hymenolepis eggs excreted in the faeces.

The success of the cure is evaluated by counting the number of eggs per gram of stool. This count should show no eggs by the end of the treatment.

In order to be certain that the parasites have been totally eliminated, the intestine of the mouse is dissected, after autopsy, in order to ensure that there are no longer any worms or that they are all dead.

The reference substances used in the in vivo test are mepacrine and helenin.

The results of the in vitro experiments are expressed in terms of LD 100 lethal doses after 24 hours, that is to say in terms of the amounts of products sufficient to kill 100% of the cestodes in 24 hours.

The results of the in vivo experiments indicate the percentage elimination of parasites obtained for the indicated amounts.

In vitro

Substance	LD 100 (24 hours)
Helenin (reference substance)	50 μg/ml
Santonin ,,	100 μg/ml
α-Hederin	100 μg/ml
90% strength extract	1 mg/ml
60% strength extract	5 mg/ml

In vivo

Substance	Dose (mg/kg)	% elimination of parasites
Mepacrine	220	1,000
Helenin	300	60
α-Hederin	400	20
90% strength extract	400	30
60% strength extract	400	60

1.2 The nematicidal activity was studied on mouse Syphacia obvelata.

The in vitro and in vivo tests are carried out in the same manner as those used for research into the taenicidal activity. The reference products in this case are santonin and helenin for the *in vitro* tests and piperazine and helenin for the *in vivo* tests.

In vitro Substance	LD 100 (24 hours)
Helenin	10 μg/ml
Santonin	100 μg/ml
α-Hederin	1 mg/ml
90% strength extract	5 mg/ml
60% strength extract	5 mg/ml

In vivo Substance	Dose (mg/kg)	% elimination of parasites
Piperazine citrate	200	90
Helenin	300	80
α-Hederin	400	10
90% strength extract	400	30
60% strength extract	400	70

1.3 The activity with respect to trematodes was studied on flukes in vitro and in vivo.

In vitro experiment:

The flukes, namely Fasciola hepatica (liver fluke) and Dicrocoelium lanceolatum (lancet fluke) are recovered, immediately after the sheep and cattle infested with the parasites have been slaughtered, by dissecting the bile ducts and hepatic ducts of these animals.

These worms are placed directly in Benex survival medium modified by Cavier, in an oven at 37°.

The products to be tested are brought into contact therewith at various concentrations and the lethal dose (LD 100) after 24 hours is determined; the reference substances are helenin and santonin.

In vivo experiment on sheep infested with the parasites:

The level of infestation of the sheep to be treated is monitored beforehand by counting the number of fluke eggs in the faeces. With the aid of a dosing gun placed at the back of the throat of the sheep, the animals are then caused to ingest 3 successive doses of the various products at 8-day intervals. The duration of the treatment is therefore 3 weeks.

Throughout the treatment period, the decrease in the number of eggs in the faeces is monitored by counting, until the eggs have disappeared. Finally, to ensure that the cure has been successful, the sheep are slaughtered and the disappearance of the flukes in the hepatic ducts and bile ducts is investigated, this being the irrefutable proof of the flukicidal activity of the products tested.

The results are expressed in the same manner as in the previous experiments:

In vitro

(a) on Fasciola hepatica

Substance	LD 100 (24 hours)
Santonin	100 μg/ml
Helenin	10 μg/ml
α-Hederin	5 μg/ml
90% strength extract	5 mg/ml
60% strength extract	1 mg/ml

(b) on Dicrocoelium lanceolatum

Substance	LD 100 (24 hours)
Santonin	50 μg/ml
Helenin	10 μg/ml
α-Hederin	10 μg/ml
90% strength extract	5 mg/ml
60% strength extract	500 μg/ml

In vivo

3 treatments of 800 mg/kg at 8-day intervals were carried out on sheep infested with lancet fluke (Dicrocoelium lanceolatum) parasites. With α-hederin, the number of eggs in the faeces decreases, whereas with the two extracts, containing 60 and 90% of hederasaponin C, the eggs totally disappear.

Monitoring after autopsy showed that the flukes had effectively disappeared or that the flukes were dead when the animals had been treated with the two 60 and 90% strength extracts.

2. *The protozoicidal activity* was studied on intestinal protozoa (amoebae) and on Trichomonas intestinalis.

The activity of the substances is studied in 2 tests:

Inhibition starting from cultures:

The minimum inhibitory concentration (MIC), that is to say the smallest amount of product which, when introduced into the culture medium before inoculation, completely stops the development of the culture after a contact time of 72 hours at 37°, is determined.

Lethal action on a 48-hour culture:

The smallest amount of product to be studied, which, when introduced into a rapidly growing, 2-day culture, is capable of killing all the protozoa (amoebae or Trichomonas) after an incubation period of 48 hours at 37°, is determined.

These 2 tests are carried out in parallel with a known reference product, namely metronidazole.

For these tests, the activity of the products was monitored by preparing retrocultures from the media in which the protozoa had been killed. These negative retrocultures confirmed the activity of the products tested.

The results are as follows:

Substance	MIC
Metronidazole	5 μg/ml
α-Hederin	50 μg/ml
90% strength extract	>10 mg/ml
60% strength extract	>10 mg/ml

Antifungal activity

The activity was studied on yeasts and dermatophytes.

1. *The activity on yeasts* (Candida albicans) was studied in vitro and in vivo.

In vitro:

The yeast Candida albicans is cultured on a doubly concentrated, liquid Sabouraud medium containing 10^6 cells in suspension.

A determined amount of this inoculum is brought into contact with decreasing doses of the dilutions of the products to be tested. The results are assessed after an incubation period of 24

The tubes in which the products have acted remain clear.

This antifungal activity is monitored by preparing retrocultures which should remain negative 72 hours after incubation in an oven at 37°.

The reference substance is amphotericin B.

In vivo:

The study is carried out on mice.

After dorsal epilation of the mice and exposure to UV in order to cause an inflammatory reaction, a subcutaneous candidiasic abscess is produced by inoculating with 0.25 ml of a Candida albicans culture diluted in a ratio of 1/10.

After two days, the treatment is commenced by dividing the mice into different batches which are to receive different concentrations of products to be tested, by means of forced ingestion with the aid of a stomach tube. This treatment is carried out in parallel with a reference product, namely amphotericin B. The treatment time is 10 days.

The success of the cure is shown by the progressive disappearance of the candidiasic abscesses.

The untreated control animals retain these abscesses for more than one month.

The results are expressed in terms of minimum inhibitory concentrations (MIC) for the in vitro test.

Substance	MIC
Amphotericin B	2.5 μg/ml
α-Hederin	500 μg/ml
90% strength extract	> 50 mg/ml
60% strength extract	> 50 mg/ml

For the in vivo test, the disappearance or non-disappearance of the subcutaneous abscess caused in mice is determined:

The abscess persists for more than one month in the case of the untreated animals.

The abscess persists in the case of animals treated with 2.5 mg/kg of amphotericin B, but a further treatment for 10 days results in the disappearance of the abscess.

The three substances of the invention, namely α-hederin and the extracts containing 90% and 60% of hederasaponin C, at a daily dose of 50 mg/kg for 10 days, lead to the disappearance of the abscesses in all cases.

2. *The activity on dermatophytes* was studied in vitro on Microsporum canis.

The dermatophyte is cultured in the following manner:

The inoculum is a 1/10 dilution of a 7-day culture in sterile water. Each tube receives 0.1 ml of this dilution, 1 ml of a doubly concentrated Sabouraud medium and 1 ml of a dilution of the products.

The antifungal activity is assessed after 7 days. The growth inhibition in the various tubes is investigated. If this inhibition is observed, a retroculture is prepared for monitoring purposes, and an assessment is made 15 days later; the negative nature of this retroculture then confirms the antifungal activity of the product.

The reference substance is amphotericin B.

The results are expressed in terms of MIC.

Substance	MIC
Amphotericin B	2.5 μg/ml
α-Hederin	50 μg/ml

The results of the previous experiments show that the substances of the invention are medicaments having an antiparasitic and antifungal activity.

The substances of the invention, namely α-hederin, the ivy extract containing 90% of hederasaponin C and the ivy extract containing 60% of hederasaponin C, can be used for the treatment of parasitic and fungal affections in human and veterinary therapy.

The substances can be presented in any form which is suitable for local, oral or parenteral administration in association with any suitable excipient, for example in the form of tablets, sugar-coated pills, capsules, solutions to be taken orally or injected, lyophilised powders, creams, lotions and the like.

By way of example, the sugar-coated pills can have the following composition:

200 mg of the extract containing 60% of hederasaponin C
20 mg of mannitol
35 mg of microcrystalline cellulose
5 mg of magnesium stearate
for a no. 1 sugar-coated pill.

CLAIMS

1. A pharmaceutical composition comprising a climbing ivy extract containing at least 60% by weight of hederasaponin C or comprising alpha-hederin, in association with a pharmaceutically acceptable excipient.

2. A composition according to claim 1, wherein the pharmaceutical composition is in the form of sugar-coated pills, a sterile injectable solution, an ointment for topical application or an eye-wash.

3. A climbing ivy extract containing at least 60% by weight of hederasaponin C for use in a method of treatment of a parasitic or fungal infection of a human being or non-human animal.

4. A method of treating a non-human animal against a fungal or parasitic infection which comprises administering to the animal a therapeutically effective amount of a climbing ivy extract containing at least about 60% by weight of hederasaponin C or comprising alpha-hederin.

5. A method according to claim 4, wherein the extract contains about 60% or at least about 90% by weight of hederasaponin C.

6. A method according to claim 4 or 5, wherein the climbing ivy extract has been obtained by a process substantially as hereinbefore described.

7. A method according to claim 4, 5 or 6 wherein the animal has an anthelmintic infection.

8. A method according to claim 4, 5 or 6 wherein the animal is infected by cestodes, nematodes or trematodes.

9. A method according to claim 4, 5 or 6 wherein sheep or cattle are treated for liver fluke or lancet fluke.

10. A process for the preparation of a climbing ivy extract containing at least 60% by weight of hederasaponin C, which process comprises leaching powdered ivy with acetone, or a solvent having a dielectric constant about the same as that of acetone, drying the powder thus leached, extracting the dried powder with substantially pure methanol, to give a solution of extracted material in methanol, concentrating the said solution, treating the resultant concentrate with neutral activated charcoal to absorb impurities thereon, separating the charcoal from the solution, concentrating the solution, adding diethyl ether, or a mixture of organic liquids which has a polarity about the same as that of diethyl ether, to the resultant concentrate in order to precipitate saponins, and recovering a precipitate comprising a climbing ivy extract containing 60% by weight of hederasaponin C, and if desired chromatographing this extract on alumina, with methanol as eluant, to give a climbing ivy extract containing more than 60% by weight of hederasaponin C.

11. A process according to claim 10 wherein the precipitate containing 60% by weight of hederasaponin C is recovered by separating the precipitate from the liquid of the concentrate, taking up the precipitate in substantially pure methanol and separating solids from the methanolic liquid.

12. A process according to claim 10 or 11 carried out to produce a climbing ivy extract containing at least 90% by weight of hederasaponin C.

13. A process according to claim 10, substantially as described in the Example.

14. A climbing ivy extract containing hederasaponin C when produced by a process claimed in any one of claims 10 to 13.

15. A pharmaceutical composition according to claim 1 or method according to claim 4, wherein the climbing ivy extract is as claimed in claim 14.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon) Ltd.—1981.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.